BINARY BAC VECTOR AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

[001] This Utility Application is based on Provisional Application 60/241,688, filed October 19, 2000, the content of which is relied upon and incorporated herein by reference in its entirety, and benefit priority under 35 U.S.C. §119(e) is hereby claimed.

GOVERNMENT SUPPORT

[002] The subject matter of this application was made with support from the United States Government (National Science Foundation Plant Science Center Grant No. 175-8300-6550-361).

FIELD OF THE INVENTION

[003] The present invention relates to methods for transferring and expressing heterologous DNA using a bacterial artificial chromosome (BAC) vector and the binary (BIN) vector.

BACKGROUND OF THE INVENTION

[004] Throughout this application various publications are referenced, many in parenthesis. Full citations for these publications are provided at the end of the Detailed Description. The

disclosures of these publications in their entireties are hereby incorporated by reference in this application.

[005] Living organisms exhibit a vast and diverse ability to perform biochemical processes leading to the synthesis of simple and complex molecules. It is often difficult to identify chemicals of interest that are synthesized by living organisms because of the limited quantities of the organism or tissues where the chemical(s) is produced. Quantity may be limited because the organisms cannot be grown in culture or propagated readily outside of their natural environment. Environmental conditions may make it difficult or impossible to collect the organism from nature. A number of standard methods now exist to extract nucleic acids from the environment. DNA copies of RNA or DNA isolated from the environment can be cloned and propagated in bacteria. It is particularly useful to clone large segments of DNA from organisms. In prokaryotes and sometimes eukaryotes, genes in the same biosynthetic or degradatative pathway are sometimes clustered. If a cluster of genes from a wild organism is introduced into a bacterium, the bacterium might express sufficient quantities of gene products from the cloned DNA to carry out the synthesis or degradation of interesting chemicals. However, identification of novel products or biochemical pathways has been hampered by the size of DNA which can be inserted into traditional cloning vectors such as bacteriophage vectors, plasmids or cosmids. Some such commonly used vectors also have the problem that they are not stable in the host, especially if they are present in high copy number.

[006] The vectors typically used for cloning large genomic sequences are: yeast artificial chromosome (YAC), bacterial artificial chromosomes (BAC), P1 or cosmids, usually requiring random subcloning in M13-like vectors. Generation of genomic libraries in YACs allows the cloning of large inserts for long-range physical mapping of complex mammalian genomes

(Burke et al., 1987). However, YACs are often unstable and also a difficult source for obtaining pure DNA in sufficient quantities for the preparation of the small-fragment libraries required for DNA sequencing (Osoegawa et al., 1998). Furthermore, many mammalian DNA sequences contain repetitive DNA sequences. Cloning of such repetitive sequences into bacteriophage vectors, plasmids and YAC vectors renders these sequences unstable (Schalkwyk et al., 1995). This results in gaps in physical genomic maps and precludes the use of these vectors as a means of propagating repetitive DNA. Furthermore, Sinden *et al.*, (1991) point to the structural instability of plasmids containing indirect repeats. As with direct repeats, this study shows that there is correlation between the size of the indirect repeat and the degree of structural instability.

[007] It would be desirable to have a method for transferring and expressing large segments of heterologous DNA in a variety of host cells including prokaryotic and non-plant eukaryotic cells.

[008] Hamilton (PCT WO 96/21725; US 5,733,744; C. Hamilton, *Gene*, 1997; the contents of which are incorporated herein by reference) discloses a vector, referred to as the BIBAC vector for *Agrobacterium*-mediated plant transformation and formation of genomic libraries. We describe here that the BIBAC vector containing heterologous DNA can be used to transform both prokaryotic and non-plant eukaryotic hosts, including yeast and fungi, and that the heterologous DNA can sometimes be expressed in these hosts. These hosts containing the BIBAC incorporating heterologous DNA can therefore be used in screens for genes for novel biosynthetic or degradative pathways and for production or degradation of various compounds.